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Critical Health Issues Associated with Review of the Scientific Criteria for Oxides of Nitrogen

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16. ABSTRACT

The Clean Air Act (CAA) requires periodic review of existing criteria that form the basis for nitrogen oxides air quality standards. These air quality criteria are to reflect the latest scientific information useful in indicating the kind and extent of all identifiable effects on public health that may be expected from the presence of nitrogen oxides in ambient air. In keeping with the requirements of the CAA, the U.S. Environmental Protection Agency is reviewing the criteria for nitrogen oxides. during the initial review, a number of critical issues germane to the review were identified and are presented here. Additional session papers provide more detailed discussion of these issues. This paper discusses specific health issues pertaining to: (1) mechanisms of action of nitrogen oxides; (2) exposure assessment; and (3) specific health effects occurring in groups of individuals considered to be at greatest risk to nitrogen oxides at ambient or near-ambient exposure levels.

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Critical Health Issues Associated with Review of the Scientific Criteria for Oxides of Nitrogen

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INTRODUCTION*

Regulatory Basis. This paper reviews the regulatory and scientific background for the current primary National Ambient Air Quality Standard (NAAQS) for nitrogen oxides (NO_x) and discusses some critical health issues germane to the current review of the scientific basis for that standard. Two sections of the Clean Air Act (CAA) govern the establishment, review, and revision of NAAQS. Section 108 (42 U.S.C. 7408) directs the Administrator of EPA to identify pollutants that may reasonably be anticipated to endanger public health and to issue air quality criteria for them. These air quality criteria are to reflect the latest scientific information useful in indicating the kind and extent of all identifiable effects on public health that may be expected from the presence of the pollutant in the ambient air. Section 109(a) (42 U.S.C. 7409) directs the EPA Administrator to propose and promulgate "primary" NAAQS for pollutants identified under section 108. Section 109(b)(1) defines a primary standard as a level of air quality, the attainment and maintenance of which in the judgment of the Administrator, based on the criteria and allowing for an adequate margin of safety, is requisite to protect the public health. Section 109(d) of the Act (42 U.S.C. 7409(d)) requires periodic review and, if appropriate, revision of existing criteria and standards. In addition, section 109(c) specifically requires the Administrator to promulgate a primary standard for nitrogen dioxide (NO_2) with an averaging time of not more than 3 h, unless no significant evidence is found that such a short-term standard is required to protect public health.

A variety of NO_x compounds and their transformation products occur naturally in the environment and also result from human activities. In addition to NO_2, nitric oxide (NO), nitrous oxide (N_2O), gaseous nitrous acid (HONO), nitric acid (HNO_3), and both nitrite and nitrate aerosols have all been found in the ambient air. The formation of nitrosamines in the atmosphere by reaction of NO_x with amines has also been suggested, but not yet convincingly demonstrated. Available scientific research on the potential health and welfare effects of NO_x compounds provides the strongest evidence linking specific adverse effects to near-ambient concentrations of NO_2. Therefore, EPA has focused its criteria reviews primarily on health and welfare effects reported to be associated with exposure to NO_2. Nitrogen dioxide is an air pollutant generated mainly by the photochemical oxidation of NO, which is emitted from a variety of mobile and stationary sources. At elevated concentrations, NO_2 can adversely affect human health, vegetation, materials, and visibility. Nitrogen oxide compounds also contribute to increased rates of acidic deposition and higher ozone concentrations in down wind rural areas.

Regulatory and Scientific Background. On April 30, 1971, EPA first promulgated NAAQS for NO_2 under section 109 of the Clean Air Act (36 FR 8186). Identical primary and secondary standards for NO_2 were set at 0.053 ppm (100 $\mu g/m^3$), averaged over one year. The scientific bases for these standards are contained in the original criteria document, "Air Quality Criteria for Nitrogen Oxides."[2] The primary standard set in 1971 was based largely on community epidemiology studies[3-5] conducted in Chattanooga, which reported respiratory effects in children exposed to low-level NO_2 concentrations over a long-term period.

In accordance with sections 108 and 109 of the Clean Air Act, in 1985 EPA completed the review of criteria upon which the existing primary and secondary NO_2 National Ambient Air Quality Standards (NAAQS) were based. Reevaluation of the Chattanooga studies in view of later information (especially regarding the accuracy of the air quality monitoring method for NO_2 used in the studies) indicated that these studies provided only limited qualitative evidence for an association between health effects and ambient exposures to NO_2. In reviewing the scientific basis for an annual standard, EPA found that the evidence showing the most serious health effects associated with chronic NO_2 exposures (e.g., emphysematous-like alterations in the lung and increased susceptibility to infection) came from animal studies conducted at concentrations well above those permitted in the ambient air by the current annual standard. Major factors impeding use of these studies for standard-setting purposes included limitations of methods for quantitatively extrapolating exposure-response results from these animal studies directly

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*This introductory text is excerpted and adapted from "Retention of the National Ambient Air Quality Standards for Nitrogen Dioxide," Final Rule.[1]

to humans. However, the seriousness of these effects, coupled with biological similarities between humans and test animals, suggested that there was some risk to human health from long-term exposure to elevated NO_2 levels. Other evidence from community epidemiology and gas stove epidemiology studies, provided some qualitative support for concluding that there is a relationship between adverse health effects and repeated, acute exposures to elevated (≥0.2-1.0 ppm) NO_2 concentrations or longer-term human exposure to near-ambient levels of NO_2. However, concern at that time for limitations associated with these studies (e.g., unreliable or insufficient monitoring data and inadequate treatment of potential confounding factors such as humidity and pollutants other than NO_2) precluded derivation of quantitative dose-response relationships at that time.

While it is not possible currently to quantify the margin of safety provided by the existing annual standard, two observations are relevant: (1) a 0.053 ppm standard was consistent with CASAC's (Clean Air Science Advisory Committee) recommendation[6] to set the annual standard at the lower end of the range (0.05 to 0.08 ppm) cited in the Office of Air Quality Planning and Standards (OAQPS) Staff Paper[7] to ensure an adequate margin of safety against long-term effects and to provide some measure of protection against possible short-term health effects; and (2) a 0.053 ppm standard would keep annual NO_2 concentrations considerably below the long-term exposure levels for which serious chronic effects have been observed in animals. Maintaining the current annual primary standard thus represented at that time a prudent public health policy choice aimed at preventing any increased chronic health risk in large, populated urban areas that attain the annual standard. On July 19, 1985, EPA announced the final decision that retained the existing annual primary and secondary standards. The decision on the need, if any, for a separate short-term standard (less than 3 h) was deferred, pending results from additional research focused on reducing uncertainties associated with evaluating short-term health effects of NO_2.

CRITICAL HEALTH ISSUES IN THE CURRENT CRITERIA DOCUMENT REVISION PROCESS

In keeping with CAA requirements, the Environmental Criteria and Assessment Office (ECAO) of EPA is reviewing and revising the criteria for NO_x. New data on air quality aspects of NO_x and health effects associated with NO_x exposure have become available since the completion of the previous NO_x air quality criteria document (AQCD).[8]

As for air quality aspects the EPA reports a mean NO_2 annual average of 0.025 ppm[9] for all U.S. monitoring sites, as compared to a corresponding value of 0.001 for isolated areas essentially unaffected by manmade NO_x emissions[10] and approximately 0.01 ppm for inhabited nonmetropolitan areas. The highest annual NO_2 average of 0.058 ppm was found in the Los Angeles (LA) area.[10] Maximum hourly NO_2 concentrations in the past have reached above 0.50 ppm in the LA area.

Indoor levels are influenced by unvented combustion sources. On average, an unvented gas cooking range adds 0.025 ppm of NO_2 to the average background concentration in a home.[11] During cooking with a gas range, peak NO_2 levels in the kitchen may reach or exceed 0.2 to 0.4 ppm. Therefore, measured exposures to NO_2 are higher for persons living in homes with gas stoves than for persons living in homes with electric stoves. About 50% of homes in the United States have gas cooking appliances; in LA more than 90% of homes have gas appliances.[12]

Besides air quality aspects, numerous health issues are being addressed as part of the current NO_x AQCD revision process. For example, as concisely summarized below, information is evaluated with regarding to the absorption and distribution of inhaled NO_2. Also being assessed are data concerning the potential induction of emphysematous-like lung alterations by chronic exposure to high NO_2 levels, as briefly noted below. Of much greater relevance for standard-setting purposes are two other critical health issues under evaluation in the AQCD process: (1) acute NO_2 exposure effects on respiratory functions in sensitive subpopulations with pre-existing respiratory diseases; and (2) NO_2 effects on increased susceptibility to respiratory illnesses in children and likely associated impacts on host respiratory defense mechanisms.

Absorption and Distribution of inhaled NO_2.

To best understand the respiratory effects of NO_2, one must understand the absorption and distribution of inhaled NO_2. When NO_2 enters the respiratory tract it first reacts with the fluids lining the cells and tissues.

Nitrogen dioxide is absorbed along the entire respiratory tract, but the major dose to tissue is predicted to be delivered to the entriacinar region, that is, the junction between the conducting and respiratory airways.[12,14] However, because NO_2 is so reactive, it is assumed that only a small amount actually reaches the blood. Little information is available on the absorption of NO_2 from the respiratory tract, but based on a study in dogs, it has been suggested that approximately 80 percent of inhaled NO_2 is absorbed by the upper respiratory tract during the resting state. The remaining 20 percent enters the lower respiratory tract where approximately 85 percent of that is absorbed.[15] Wagner[16] reported similar results in healthy humans exposed to a mixture of NO/NO_2 containing 0.29 to 7.2 ppm NO_2 for and unspecified period of time. The subjects retained 81 to 90 percent of the inhaled dose.

Emphysema and NO_2.

The potential for emphysematous-like alterations in the lung in relation to chronic NO_2 exposure represents a serious health effect. Based on the currently accepted definition of emphysema for an animal model (abnormal state of the lungs in which there is enlargement of the airspaces distal to the terminal bronchiole), a number of experimental studies have demonstrated that chronic exposure (4 to 100 weeks) to NO_2 at levels >12 ppm (much greater than ambient levels) can produce emphysematous lesions. However, emphysematous effects have not been seen in experimental animals exposed to levels <12 ppm NO_2. Thus, it is not clear what the potential for emphysema in humans would be at ambient NO_2 concentrations.

Effects of NO_2 on Respiratory Function in Sensitive Subpopulations

Aggravation of Existing Respiratory Disease. In normal subjects there has been no clear evidence of change in lung volumes, flow-volume characteristics of the lung, or in airway resistance at NO_2 exposure concentrations below 1.0 ppm.[8] Subjects with asthma who have airway hyperresponsiveness to a variety of chemical and physical stimuli are considered one of the potentially most NO_2-responsive groups in the population. The physiological endpoint which appears to be the most sensitive indicator of response in asthmatics examined to date is a change in airway responsiveness. The study that initially raised interest about airway reactivity and NO_2 exposure was that of Orehek et al.[17], in which some asthmatics apparently showed increased airway reactivity to carbachol inhalation challenge after exposure to 0.1 ppm NO_2 for 1 h. The validity of this conclusion however has been questioned.[8,18]

Controlled human exposure studies have examined changes in airway responsiveness in asthmatics to cold air, methacholine, SO_2, carbachol, and histamine following short-term NO_2 exposure. In several studies, airway responsiveness in exercising asthmatics exposed from 20 to 120 mins was increased in the exposure range of 0.2 to 0.5 ppm[19-24], while in other studies[18,25,26] it was not. After a sequential exposure, Rubinstein et al.[27] concluded that short term exposure (30 mins) of exercising asthmatics to 0.3 ppm NO_2 did not potentiate airway responsiveness to inhaled SO_2. In normal subjects, airway responsiveness to methacholine was increased after NO_2 exposures for 1 h at concentration of 2.0 ppm.[28] Furthermore, there have been no observations[21,25,26] of increased airway responsiveness for exposure for 20 to 110 mins at concentrations in excess of 0.5 ppm, up to and including 3.0 ppm. In the case of many inhaled toxic substances, there is a concentration-response relationship in which the response increases with the concentration. The absence of a graded response to NO_2 with increasing concentration cannot be readily explained at this time. Nevertheless, it is unlikely that the same observation would be made in several independent studies merely by chance. There is a possibility that a portion of the variability in response to NO_2 may be attributed to differences in severity of asthma of study subjects. The large variation in baseline performance among asthmatic subjects may be responsible for much of the inconsistency in reported findings on NO_2 studies.

In patients with chronic obstructive lung disease, Bauer et al.[29] and Morrow and Utell[30] have observed decreased lung function (FVC, FEV_1) after exposure to 0.30 ppm NO_2 for 30 mins. Linn et al.[31] and Von Nieding and Wagner[32] show that in COPD (chronic obstructive pulmonary disease) patients exposed to NO_2 levels in excess of 1.6 to 2.0 ppm NO_2 for short duration exposures (5-60 mins) causes a concentration-dependent increase in airway resistance. Asthmatics and COPD patients appear to be sensitive subpopulations.

These studies provide important data on the issue of increased airway responsiveness in sensitive subpopulations. For certain subpopulations such as asthmatics, exposure to 0.3 ppm NO_2 for 20 to 120 mins might be clinically important and 0.3 ppm NO_2 might be considered as a Lowest Observed Effect Level. However, the

issue of a nonmonotonic dose-response relationship needs more verification. Since a short-term exposure may produce a response, the issue of a need for a short-term standard is raised. Data are needed to establish a firmer basis for a determination of NO_2 levels related to responses in these sensitive subpopulations. Studies are needed that examine repeated exposures, multihour exposures and repeated exposures on several consecutive days. An improved understanding of the mechanisms that produce the apparent increase in airway reactivity will be necessary before the above response pattern can be understood.

Respiratory Infection and Effects of NO_2 on Host Respiratory Defense Systems

Increase in the Rate and/or Severity of Respiratory Illness. Respiratory illness and associated symptoms are an important public health concern. While many microorganisms are implicated in respiratory infection, viruses represent a major cause of respiratory disease. In a viral respiratory infection, viral replication results in cell death and after sufficient numbers of cells are damaged, the function of the tissue is impaired, which results in the signs and symptoms of respiratory illness.[33] An increase in the rate and/or the severity of respiratory infection is the more specific endpoint under consideration. Several studies have examined in depth the topic of viral infection in children and provide a level of understanding of the process.[34-36] Studies of concern here, however, examine symptoms of respiratory infection in children exposed to NO_2. Samet et al.[37] state that findings potentially relating NO_2 exposure to respiratory illness indicate that the magnitude of the NO_2 effect at concentrations encountered in most U.S. homes is likely to be small, but notes that even a small effect of NO_2 exposure could potentially be of public health concern because more than half of U.S. homes have gas cooking stoves and childhood respiratory illness is extremely common.

Several epidemiological studies provide evidence that repeated exposure to NO_2 increases respiratory illness in children. A key endpoint is NO_2 exposure relative to an increase in the respiratory illness rate in young children, especially those under two years of age. Ware et al.[38] studied a large cohort in six cities in the United States over the period 1974 to 1979 and estimated the effects of gas stoves vs. electric stoves in homes on the rate of serious respiratory illness in children under two. The odds ratio approached statistical significance. Dockery et al.[39] examined a second large cohort from the same six cities during the period 1983 to 1986 and reported that the health endpoint of doctor-diagnosed respiratory illness prior to age two was marginally significant. Melia et al.[40] studied a large cohort of children in 28 randomly selected areas of England and Scotland and reported a trend for increased respiratory symptoms in homes with gas stoves. In a later study[41], Melia et al. obtained indoor NO_2 measurements from 66 percent of the homes of 808 school children aged six to seven years in Middleborough, an urban area of North England. For the six to seven-year-old children living in gas stove homes, there appeared to be an increase of respiratory illness associated with increasing levels of NO_2 in their bedrooms. Other studies[42-46] also examine the relationship to respiratory illness of exposure to NO_2 either with direct measurement or by surrogate (gas stove vs. electric). While the evidence from these studies appears mixed, most studies show a positive trend, though not always significantly, of increases of respiratory illness in children associated with long-term exposure to NO_2.

The confidence that can be ascribed to the strength of the epidemiologic studies relating NO_2 exposure to an increased rate of respiratory disease is determined by several factors. Two key factors are the adequacy of the exposure estimate and the health effect measure.

Many epidemiological studies evaluating the potential of respiratory effects of NO_2 used surrogate indicators of NO_2 exposure in the evaluation. Most commonly this consisted of the presence of an emission source of NO_2 in the home, such as a gas stove, versus the lack of such a source (an electric stove). This allows the comparison of health effects where other data establish that differences in exposure to NO_2 would occur between the two situations. This is not a precise exposure estimate method and has the potential for misclassification error. Random misclassification reduces the magnitude of the observed association from the value that would be found if the exposure of subjects was correctly estimated.[47,48] However, several of these studies may contain useful information even though exposure surrogates (gas stoves versus electric) are imprecise and may lower estimates of effect. An issue is how much confidence can be put in these studies quantitatively when the exposure estimates of these studies are supplemented by data from exposure studies that relate NO_2 levels to homes with and without gas stoves. Some studies provided more direct assessment of exposures of the subjects studied with measurement of NO_2

concentrations for a part of the study.[39,41] More recently, improved exposure measures have been incorporated in other studies with models that provide more precise estimates of exposure that improve the strength of that aspect of the studies.[49-53] The exposure estimates in such studies are based on averages of one to two week estimates related to the Palmes tubes integrating concentration over that time period or other measures averaged over similar or longer time periods. A further consideration is the evaluation of the potential difference between exposure to a series of peak concentration levels as compared to lower baseline background levels and their relation to health effects. Information on activity patterns is needed for estimates of actual exposures.

Measurement of the health effect is another important issue for epidemiologic studies examining relationships between exposure to NO_2 and increased acute respiratory disease and infection. One must consider the appropriateness of the instrument used and the health effect examined to determine if they actually measure the aspect of respiratory illness being considered. The studies described above have not vigorously examined infection parameters such as isolation of the infectious agent during the symptomatic episode and seroconversion.[54] Additionally in studies of respiratory disease in children,[55] without a pollutant effect, nasal pulmonary reflexes may contribute to lower respiratory tract abnormalities with demonstrated nasal symptoms but the absence of direct infection. However, several studies of respiratory infection in children,[34-36] that are unrelated to NO_2 exposures, examine parameters of infection such as isolation of viruses during symptomatic episodes. These studies relate infection to the symptoms recorded. The similarity of some of the symptoms in the epidemiology studies of respiratory infection studied in relation to NO_2 exposure could be considered appropriate enough then to relate the symptoms to incidence of respiratory illness, if not to infectious episodes. Evidence from ongoing studies such as the Albuquerque Infant Study[49] should add further to our understanding. In general, the instrument used and health effect studied should be examined for each study to evaluate their appropriateness as outcome measures.

The above epidemiological investigations on NO_2 relationships to respiratory illness have been viewed as having methodological limitations and producing somewhat mixed results.[37,56] Some specific studies[49] are underway that are designed to address some of the methodological limitations of earlier studies. Other data bases are also appearing that provide some improvement over earlier studies. Also, looking at these studies as a group may provide a fuller perspective concerning their meaning since it allows examination of consistency between studies and the strength of the total data base. The strength and consistency of the epidemiologic database relating NO_2 exposure to respiratory disease endpoints is a key issue to be addressed in the current NO_2 NAAQS criteria review.

Effects of NO_2 on Respiratory Host Defense System. In addition to ensuring an adequate and efficient means of gas exchange, the respiratory system also has several defense mechanisms against inhaled infectious and chemical agents. Host defense mechanisms consist of nonspecific and specific components. The nonspecific aspects include mucociliary clearance and alveolar macrophages while the specific include antibodies and lymphocytes. The immune system functions through a sequence of events, starting with the nonspecific components followed by responses by the specific components. The immune system is a principal factor in the interaction with infectious agents such as viruses and in the host's ability to contain and/or eradicate the establishment of infection. Specific T cell-mediated immune mechanisms are important in augmenting host defenses of the lung.[57] T lymphocytes ($CD4^+$ and $CD8^+$) augment resistance to intracellular microbial organisms and lyse virally infected cells. To an extent, an increase of reported respiratory symptoms in some epidemiology studies may be an indication of the ability of the respiratory host-defense mechanism to either overcome an infection or limit its severity. NO_2 may affect the immune system in such a way that one or several aspects of the immune system do not function at a level sufficient to limit the extent or occurrence of infection.

Based on available information, exposure to NO_2 impairs one or more defense mechanisms, leaving the host more susceptible to respiratory infections and diseases. One of the first lines of defense against foreign agents entering the respiratory tract is the mucociliary clearance system, responsible for the removal of particles from the conducting airways of the lungs.[58] The effectiveness of this system is dependent on the integrity of the cilia and respiratory epithelia, properties of the mucus and the rate of mucus transport. In some of the earlier studies on the effects of NO_2 exposure on mucociliary transport, acute exposure to NO_2 at concentrations significantly higher than those routinely found in the environment ($\geq$2 ppm) produced a reduction in the number and activity of the cilia,

morphological changes in the cilia, and decreased mucociliary velocity.[59-62] More recently, however, losses of cilia and other structural effects were reported in rats exposed to 0.5 ppm over a seven month period.[63]

As a foreign agent moves beyond the mucociliary region, and enters the gaseous exchange region of the lung, host defenses are provided by the alveolar macrophage which acts to remove and/or kill viable particles and thereby maintain sterility against infectious agents and remove damaged and dead cells. Exposure to NO_2 has produced a variety of effects on alveolar macrophages including decreased viability and an increase in number of macrophages and volume of alveolar tissue. Generally these effects are noted after animals have been exposed to levels of NO_2 exceeding those encountered in the environment (0.5 ppm with 2 daily spikes to 1.5 ppm or continuous exposure to $\geq$2.7 ppm for 10 days).[64-67] While decreases in the phagocytic ability of alveolar macrophages have been reported in rats, hamsters, and rabbits exposed to levels of $NO_2 \geq 4$ ppm[65,68-70] which are substantially above those that occur in ambient air, Schlesinger[71] reported that a 13-day intermittent exposure for 2h/day to 0.3 ppm NO_2 decreased alveolar macrophage functional capacity.

Controlled human exposure studies have examined alveolar macrophage function. Frampton et al.[72] exposed normal human volunteers to NO_2. Alveolar macrophages, obtained by bronchoalveolar lavage three and one half h after exposure of subjects to continuous 0.60 ppm NO_2, tended to inactivate influenza virus *in vitro* less effectively than cells collected after air exposure. Pinkston et al.[73] exposed human alveolar macrophages *in vitro* to NO_2 and were unable to demonstrate a direct toxic effect of NO_2 on viability or any NO_2-induced change in release of immunoregulatory molecules.

In addition to the above functional changes to alveolar macrophages, NO_2 exposure has also been reported to produce structural changes in the macrophages. Such changes, which include loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas, have been reported in mice continuously exposed to 2 ppm NO_2 or 0.5 ppm with a 1-h spike to 2 ppm 5 days/week for 21 weeks. Exposure to lower levels of NO_2 (0.5 ppm or 0.1 ppm with a 1-h spike to 1 ppm for 24 weeks) did not produce any morphological changes over that of the controls.[74] NO_2-induced structural changes to alveolar macrophages have also been reported in rabbits and rats with increasing NO_2 exposure levels.[75,76]

Few studies are available that evaluate the effects of NO_2 on the immune system; however, there is some indication that exposure to NO_2 suppresses the immune response and that the effect is concentration- and time-dependent. For example, a significant suppression of humoral antibody response has been reported in experimental animals acutely exposed for 12 hrs. to levels of NO_2 of $\geq$5 ppm.[77,78] When the exposure concentration was lowered (0.4 ppm) and the exposure time was increased (4 weeks) similar effects were reported in the humoral antibody response.[79] Richters and Damji[80] reported that exposure to as little as 0.25 ppm for 7 weeks significantly suppressed the number of mature spleen helper T and cytotoxic T lymphocytes. The cause of the suppression is not clear. Exposure to 0.4 ppm NO_2 for 8 h resulted in decreased numbers of T lymphocyte, T-helper/inducer lymphocytes, and T-cytotoxic/suppressor lymphocytes in mice.[81]

Recent controlled human exposure studies have examined the effects of NO_2 on pulmonary host defense systems. Goings et. al.[82] reported a trend toward an elevated rate of infection, though not statistically significant, in a study examining the effect of NO_2 exposure on the infectivity rate of live attenuated influenza A/Korea/reassortment virus. The frequencies of infection in two of the four groups exposed to NO_2 were higher than the 56 to 73% infection rates observed in previous studies in healthy adult volunteers with the same dose of influenza A virus. Frampton et al.[83] observed that continuous exposure for 3 h to 0.60 ppm NO_2 may transiently increase levels of antiprotease alpha-2-macroglobulin (α_2M) in BAL fluids presumably through local release. While serving as an indicator of changes in protease-antiprotease balance, alterations in α_2M in the alveolus may have significance for local immunoregulation. Changes in α_2M may alter alveolar macrophage defenses against infection. These findings suggest, but do not prove, that NO_2 may play a role in increasing the susceptibility of adults to respiratory virus infections.

Animal infectivity studies present key data relating exposure to NO_2 and effects on host defense mechanisms. In these studies, animals were exposed to varying concentrations of NO_2 followed by exposure to an aerosol of an

infectious agent. Mortality was used as the endpoint. The infectivity model in which air and NO_2-exposed mice are challenged with a viable microbial aerosol and mortality is measured is the most sensitive. A 6-month exposure to 0.5 ppm NO_2 increased bacterial-induced mortality[84] and a 39-day exposure to 0.5 ppm NO_2 increased influenza-induced mortality.[85] In acutely exposed mice[86] 2 ppm NO_2 for 3 h produced a bacterial-induced increase in mortality. Although this would not be a suitable endpoint when evaluating the effects of any agent in humans, the results indicate that NO_2 increased microbial-induced mortality by impairing the host's ability to defend the respiratory tract from infections agents. Recently, Parker et al.[87] reported on an infectivity model designed to evaluate the effects of NO_2 on nonfatal respiratory disease. In that study, two strains of mice, C57BL-6N and C3H-HeN that differ in their susceptibility to murine respiratory mycoplasmosis were exposed to 0, 5, or 10 ppm NO_2 and to *Mycoplasma pulmonis*. Nitrogen dioxide decreased the intrapulmonary removal (killing) of the microorganisms in both strains of mice, in a concentration-related manner without a decrease in the number of alveolar macrophages. There was no nitrogen dioxide-induced effect on the levels of IgM antibody specific to *M. pulmonis* in exposed mice. Exposure to NO_2 was found to increase the severity of the mycoplasma-induced lesions but not found to increase the susceptibility of the mice to the infection. These animal studies clearly demonstrate that acute and chronic exposures to NO_2 increase susceptibility to viral, mycoplasma, and bacterial infections and the effects were concentration- and time-dependent, species dependent, and infectious agent dependent.[83,87-95]

While animal infectivity models have clearly shown that exposure to NO_2 increases susceptibility to infection, they may not be sensitive enough to detect changes in lung defenses that might occur during exposure to lower concentrations (ambient and lower) of NO_2.[96] A more detailed analysis of various components of the lung defense apparatus may be necessary to detect adverse effects of low NO_2 concentration. Further important information regarding subtle but important effects may come from studies of antiviral defense mechanisms such as Natural Killer (NK) cells, cytotoxic lymphocytes and local and systemic interferon responses.[96]

Animal toxicological and human clinical studies of host defenses form a basis that helps explain the relationship seen in epidemiology studies between exposure to NO_2 and an increase in frequency and severity of respiratory symptoms and/or infections in humans. The strength of various parts of the database that substantiate the scientific basis for the relationship seen in epidemiology studies between respiratory disease and exposure to NO_2 is a key issue to be addressed in the current NO_x criteria review.

SUMMARY

In 1985, the EPA Administrator announced the decision to retain the primary annual standard of 0.053 ppm for NO_2 because it was both necessary and sufficiently prudent to protect public health against chronic effects with an adequate margin of safety and because it provided some measure of protection against possible short-term health effects. In keeping with Clean Air Act requirements, ECAO is reviewing and revising the criteria for oxides of nitrogen. As part of this the current criteria review several critical issues related to health effects are being evaluated, as briefly summarized in Table 1. This includes evaluation of evidence bearing on the possible need for a separate short-term (less than 3 h) NO_2 standard. Currently ongoing research studies, when published, may add important results to help address these issues. However, future research will still be needed to fully evaluate the health effects of NO_2.

TABLE 1. CRITICAL ISSUES RELATED TO HEALTH EFFECTS OF EXPOSURE TO NO_2

Effects of Nitrogen Dioxide on Respiratory Functions and Disease

1. Emphysematous potential in humans related to chronic low NO_2 exposure concentrations.

2. Basis for determination of NO_2 levels related to response in the potentially sensitive subpopulations, asthmatics and COPD patients.

   a. Endpoint of bronchial reactivity with responses to short-term exposures (<3 hrs) at around 0.3 ppm.

   b. Hypothesis of a nonmonotonic dose-response relationship.

3. Relationship between NO_2 exposures and increased rate and/or severity of respiratory disease and symptoms.

   a. Young children as a susceptible population.

   b. Direct evidence of the relationship between exposure to NO_2 and increased respiratory infection in humans. Evaluation of the appropriateness of health endpoint studied.

   c. Evaluation of misclassification error in exposure estimates used in epidemiology studies.

   d. Adequacy of exposure estimates for epidemiology studies to include method of estimation, evaluation of background levels vs. peak levels, and representativeness of exposures.

   e. Strength and consistency of the epidemiology database relating exposure to NO_2 to respiratory illness.

4. Effects of NO_2 on Respiratory Host-Defense Systems and their Relationship to Respiratory Infection and the Immune System

   a. Strength of the data base that substantiates the scientific basis for the relationship seen in epidemiology studies between respiratory disease and exposure to NO_2.

   b. Exposure-response effects on host defense mechanisms including mucociliary clearance systems, alveolar macrophages, and the immune system.

REFERENCES

1. Federal Register. (1985) Retention of the national ambient air quality standards for nitrogen dioxide: final rule. F. R. (June 19) 50: 25532-25545.

2. U. S. Environmental Protection Agency. (1971) Air quality criteria for nitrogen oxides. Washington, DC: U. S. Environmental Protection Agency, Air Pollution Control Office; EPA report no. AP-84. Available from: NTIS, Springfield, VA; PB-197333/BE.

3. Shy, C. M.; Creason, J. P.; Pearlman, M. E.; McClain, K. E.; Benson, F. B.; Young, M. M. (1970) The Chattanooga school children study: effects of community exposure to nitrogen dioxide. I. Methods, description of pollutant exposure, and results of ventilatory function testing. J. Air Pollut. Control Assoc. 20: 539-545.

4. Shy, C. M.; Creason, J. P.; Pearlman, M. E.; McClain, K. E.; Benson, F. B.; Young, M. M. (1970) The Chattanooga school children study: effects of community exposure to nitrogen dioxide. II. Incidence of acute respiratory illness. J. Air Pollut. Control Assoc. 20: 582-588.

5. Pearlman, M. E.; Finklea, J. F.; Creason, J. P.; Shy, C. M.; Young, M. M.; Horton, R. J. M. (1971) Nitrogen dioxide and lower respiratory illness. Pediatrics 47: 391-398.

6. Friedlander, S. K. (1982) CASAC review and closure of the OAQPS staff paper for nitrogen oxides [memorandum to Anne M. Gorsuch]. July 6.

7. U. S. Environmental Protection Agency. (1982) Review of the national ambient air quality standards for nitrogen oxides: assessment of scientific and technical information; OAQPS staff paper. Research Triangle Park, NC: Office of Air Quality Planning and Standards; EPA report no. EPA-450/5-82-002. Available from: NTIS, Springfield, VA; PB83-132829.

8. U. S. Environmental Protection Agency. (1982) Air quality criteria for oxides of nitrogen. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; EPA report no. EPA-600/8-82-026. Available from: NTIS, Springfield, VA; PB83-131011.

9. U. S. Environmental Protection Agency. (1989) National air quality and emissions trends report, 1987. Research Triangle Park, NC: Office of Air Quality Planning and Standards; report no. EPA/450/4-89/001.

10. U. S. Environmental Protection Agency. (1987) National air quality and emissions trends report, 1985. Research Triangle Park, NC: Office of Air Quality Planning and Standards; EPA report no. EPA-450/4-87-001. Available from: NTIS, Springfield, VA; PB87-180352.

11. Spengler, J. D.; Duffy, C. P.; Letz, R.; Tibbitts, T. W.; Ferris, B. G., Jr. (1983) Nitrogen dioxide inside and outside 137 homes and implications for ambient air quality standards and health effects research. Environ. Sci. Technol. 17: 164-168.

12. U. S. Bureau of the Census. (1982) Census of population and housing. Supplementary report: provisional estimates of social, economic and housing characteristics - states and selected standard metropolitan statistical areas. Washington, DC: U. S. Government Printing Office.

13. Miller, F. J.; Overton, J. H.; Myers, E. T.; Graham, J. A. (1982) Pulmonary dosimetry of nitrogen dioxide in animals and man. In: Schneider, T.; Grant, L., eds. Air pollution by nitrogen oxides: proceedings of the US-Dutch international symposium; May; Maastricht, The Netherlands. Amsterdam, The Netherlands: Elsevier Scientific Publishing Company; pp. 377-386. (Studies in environmental science 21).

14. Overton, J. H., Jr. (1984) Physicochemical processes and the formulation of dosimetry models. In: Miller, F. J.; Menzel, D. B., eds. Fundamentals of extrapolation modeling of inhaled toxicants: ozone and nitrogen dioxide. Washington, DC: Hemisphere Publishing Corporation; pp. 93-114.

15. Kleinman, M. T.; Mautz, W. J. (1987) The effects of exercise on respiratory tract dosimetry for inhaled gaseous pollutants. Presented at: 80th annual meeting of the Air Pollution Control Association; June; New York, NY. Pittsburgh, PA: Air Pollution Control Association; paper no. 87-33.5.

16. Wagner, H.-M. (1970) Absorption von NO und NO_2 in MIK- und MAK-Konzentrationen bei der Inhalation [Absorption of NO and NO_2 in mik- and mac-concentrations during inhalation]. Staub Reinhalt. Luft 30: 380-381.

17. Orehek, J.; Massari, J. P.; Gayrard, P.; Grimaud, C.; Charpin, J. (1976) Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J. Clin. Invest. 57: 301-307.

18. Hazucha, M. J.; Ginsberg, J. F.; McDonnell, W. F.; Haak, E. D., Jr.; Pimmel, R. L.; Salaam, S. A.; House, D. E.; Bromberg, P. A. (1983) Effects of 0.1 ppm nitrogen dioxide on airways of normal and asthmatic subjects. J. Appl. Physiol.: Respir. Environ. Exercise Physiol. 54: 730-739.

19. Avol, E. L.; Linn, W. S.; Peng, R. C.; Valencia, G.; Little, D.; Hackney, J. D. (1988) Laboratory study of asthmatic volunteers exposed to nitrogen dioxide and to ambient air pollution. Am. Ind. Hyg. Assoc. J. 49: 143-149.

20. Bylin, G.; Lindvall, T.; Rehn, T.; Sundin, B. (1985) Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Eur. J. Respir. Dis. 66: 205-217.

21. Bylin, G.; Lindvall, T.; Rehn, T.; Sundin, B. (1987) Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Experientia Suppl. 51: 227-230.

22. Kleinman, M. T.; Bailey, R. M.; Linn, W. S.; Anderson, K. R.; Whynot, J. D.; Shamoo, D. A.; Hackney, J. D. (1983) Effects of 0.2 ppm nitrogen dioxide on pulmonary function and response to bronchoprovocation in asthmatics. J. Toxicol. Environ. Health 12: 815-826.

23. Bauer, M. A.; Utell, M. J.; Morrow, P. E.; Speers, D. M.; Gibb, F. R. (1986) Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. Am. Rev. Respir. Dis. 134: 1203-1208.

24. Mohsenin, V. (1987) Airway responses to nitrogen dioxide in asthmatic subjects. J. Toxicol. Environ. Health 22: 371-380.

25. Roger, L. J.; Horstman, D. H.; McDonnell, W. F.; Kehrl, H.; Ives, P. J.; Seal, E.; Chapman, R.; Massoro, E. (1989) Pulmonary function, airway responsiveness, and respiratory symptoms in asthmatics following exercise in NO_2. Toxicol. Ind. Health: (in press).

26. Linn, W. S.; Shamoo, D. A.; Avol, E. L.; Whynot, J. D.; Anderson, K. R.; Venet, T. G.; Hackney, J. D. (1986) Dose-response study of asthmatic volunteers exposed to nitrogen dioxide during intermittent exercise. Arch. Environ. Health 41: 292-296.

27. Rubinstein, I.; Bigby, B. G.; Reiss, T. F.; Boushey, H. A., Jr. (1990) Short-term exposure to 0.3 ppm nitrogen dioxide does not potentiate airway responsiveness to sulfur dioxide in asthmatic subjects. Am. Rev. Respir. Dis. 141: 381-385.

28. Mohsenin, V. (1988) Airway responses to 2.0 ppm nitrogen dioxide in normal subjects. Arch. Environ. Health 43: 242-246.

29. Baner, M. A.; Utell, M. J.; Speers, D. M.; Gibb, F. R.; Morrow, P. E. (1987) Effects of 0.30 ppm nitrogen dioxide on lung function and breathing patterns in subjects with chronic obstructive lung disease (COPD). Am. Rev. Respir. Dis. 135(suppl.): A58.

30. Morrow, P. E.; Utell, M. J. (1989) Responses of susceptible subpopulations to nitrogen dioxide. Cambridge, MA: Health Effects Institute; research report no. 23.

31. Linn, W. S.; Shamoo, D. A.; Spier, C. E.; Valencia, L. M.; Anzar, U. T.; Venet, T. G.; Avol, E. L.; Hackney, J. D. (1985) Controlled exposure of volunteers with chronic obstructive pulmonary disease to nitrogen dioxide. Arch. Environ. Health 40: 313-317.

32. von Nieding, G.; Wagner, H. M. (1979) Effects of NO_2 on chronic bronchitics. EHP Environ. Health Perspect. 29: 137-142.

33. Douglas, R. G., Jr. (1986) Pathogenetic mechanisms in viral respiratory tract infections. In: Sande, M. A.; Hudson, L. D.; Root, R. K., eds. Respiratory infections. New York, NY: Churchill Livingstone, Inc.; pp. 25-46.

34. Wright, A. L.; Taussig, L. M.; Ray, C. G.; Harrison, H. R.; Holberg, C. J. (1989) The Tucson children's respiratory study: II. lower respiratory tract illness in the first year of life. Am. J. Epidemiol. 129: 1232-1246.

35. Glezen, W. P.; Taber, L. H.; Frank, A. L.; Kasel, J. A. (1986) Risk of primary infection and reinfection with respiratory syncytial virus. Am. J. Dis. Child. 140: 543-546.

36. Dingle, J. H.; Badger, G. F.; Jordan, W. S., Jr. (1964) Etiology of the common respiratory diseases. In: Illness in the home: a study of 25,000 illnesses in a group of Cleveland families. Cleveland, OH: The Press of Western Reserve University; pp. 129-161.

37. Samet, J. M.; Marbury, M. C.; Spengler, J. D. (1987) Respiratory effects of indoor air pollution. Presented at: Fourth annual Aspen allergy conference; July 1986; Aspen, CO. J. Allergy Clin. Immunol. 79: 685-700.

38. Ware, J. H.; Dockery, D. W.; Spiro, A., III; Speizer, F. E.; Ferris, B. G., Jr. (1984) Passive smoking, gas cooking, and respiratory health of children living in six cities. Am. Rev. Respir. Dis. 129: 366-374.

39. Dockery, D. W.; Spengler, J. D.; Neas, L. M.; Speizer, F. E.; Ferris, B. G., Jr.; Ware, J. H.; Brunekreef, B. (1989) An epidemiologic study of respiratory health status and indicators of indoor air pollution from combustion sources. In: Combustion processes and the quality of the indoor environment, proceedings of the AWMA specialty conference; September 1988. Pittsburgh, PA: Air and Waste Management Association; pp. 262-271. (AWMA specialty conference no. TR-15).

40. Melia, R. J. W.; Florey, C. V.; Altman, D. G.; Swan, A. V. (1977) Association between gas cooking and respiratory disease in children. Br. Med. J. 2: 149-152.

41. Melia, R. J. W.; Florey, C. du V.; Chinn, S.; Goldstein, B. D.; Brooks, A. G. F.; John, H. H.; Clark, D.; Craighead, I. B.; Webster, X. (1980) The relation between indoor air pollution from nitrogen dioxide and respiratory illness in primary schoolchildren. Clin. Respir. Physiol. 16: 7P-8P.

42. Ogston, S. A.; Florey, C. du V.; Walker, C. H. M. (1985) The Tayside infant morbidity and mortality study: effect on health of using gas for cooking. Br. Med. J. 290: 957-960.

43. Ekwo, E. E.; Weinberger, M. M.; Lachenbruch, P. A.; Huntley, W. H. (1983) Relationship of parental smoking and gas cooking to respiratory disease in children. Chest 84: 662-668.

44. Houthuijs, D.; Remijn, B.; Brunekreef, B.; de Koning, R. (1987) Exposure to nitrogen dioxide and tobacco smoke and respiratory health of children. In: Seifert, B.; Esdorn, H.; Fischer, M.; Rueden, H.; Wegner, J., eds. Indoor air '87: proceedings of the 4th international conference on indoor air quality and climate; v. 1, volatile organic compounds, combustion gases, particles and fibres, microbiological agents; August; Berlin, Federal Republic of Germany. Berlin, Federal Republic of Germany: Institute for Water, Soil and Air Hygiene; pp. 463-467.

45. Keller, M. D.; Lanese, R. R.; Mitchell, R. I.; Cote, R. W. (1979) Respiratory illness in households using gas and electricity for cooking. I. Survey of incidence. Environ. Res. 19: 495-503.

46. Keller, M. D.; Lanese, R. R.; Mitchell, R. I.; Cote, R. W. (1979) Respiratory illness in households using gas and electricity for cooking: II. symptoms and objective findings. Environ. Res. 19: 504-515.

47. Shy, C. M.; Kleinbaum, D. G.; Morgenstern, H. (1978) The effect of misclassification of exposure status in epidemiological studies of air pollution health effects. Bull. N. Y. Acad. Med. 54: 1155-1165.

48. Ozkaynak, H.; Ryan, P. B.; Spengler, J. D.; Laird, N. M. (1984) Bias due to misclassification of personal exposures in epidemiologic studies of indoor and outdoor air pollution. In: Berglund, B.; Lindvall, T.; Sundell, J., eds. Indoor air: proceedings of the 3rd international conference on indoor air quality and climate, v. 3, sensory and hyperreactivity reactions to sick buildings; August; Stockholm, Sweden. Stockholm, Sweden: Swedish Council for Building Research; pp. 377-384. Available from: NTIS, Springfield, VA; PB85-104206.

49. Samet, J. M.; Spengler, J. D. (1989) Nitrogen dioxide and respiratory infection: pilot investigations. Cambridge, MA: Health Effects Institute; research report no. 28.

50. Leaderer, B. P.; Zagraniski, R. T.; Berwick, M.; Stolwijk, J. A. J. (1987) Predicting NO_2 levels in residences based upon sources and source use: a multivariate model. Atmos. Environ. 21: 361-368.

51. Berwick, M. (1987) Lower respiratory symptoms in children associated with nitrogen dioxide exposure [dissertation]. New Haven, CT: Yale University. Available from: University Microfilms, Ann Arbor, MI; publication no. 87-29,173.

52. Harlos, D. P.; Marbury, M.; Samet, J.; Spengler, J. D. (1987) Relating indoor NO_2 levels to infant personal exposures. Atmos. Environ. 21: 369-376.

53. Marbury, M. C.; Harlos, D. P.; Samet, J. M.; Spengler, J. D. (1988) Indoor residential NO_2 concentrations in Albuquerque, New Mexico. JAPCA 38: 392-398.

54. Jakab, G. J. (1988) Modulation of pulmonary defense mechanisms against viral and bacterial infections by acute exposures to nitrogen dioxide. Cambridge, MA: Health Effects Institute; research report number 20.

55. Taussig, L. M.; Busse, W. W.; Lemen, R. J.; Ram, J. S. (1988) NHLBI workshop summary. Models of infectious airway injury in children. Am. Rev. Respir. Dis. 137: 979-984.

56. Samet, J. M.; Tager, I. B.; Speizer, F. E. (1983) The relationship between respiratory illness in childhood and chronic air-flow obstruction in adulthood. Am. Rev. Respir. Dis. 127: 508-523.

57. Kaltreider, H. B. (1986) Immune defenses of the lung. In: Sande, M. A.; Hudson, L. D.; Root, R. K., eds. Respiratory infections. New York, NY: Churchill Livingstone, Inc.; pp. 47-70.

58. Proctor, D. F.; Andersen, I.; Lundquist, G. (1977) Nasal mucociliary function in humans. In: Brain, J. D.; Proctor, D. F.; Reid, L. M., eds. Respiratory defense mechanisms (in two parts): part I. New York, NY: Marcel Dekker, Inc.; pp. 427-452. (Lung biology in health and disease: v. 5).

59. Stephens, R. J.; Freeman, G.; Evans, M. J. (1972) Early response of lungs to low levels of nitrogen dioxide: light and electron microscopy. Arch. Environ. Health 24: 160-179.

60. Azoulay, E.; Soler, P.; Blayo, M. C. (1978) The absence of lung damage in rats after chronic exposure to 2 ppm nitrogen dioxide. Bull. Eur. Physiopathol. Respir. 14: 311-325.

61. Schiff, L. J. (1977) Effect of nitrogen dioxide on influenza virus infection in hamster trachea organ culture. Proc. Soc. Exp. Biol. Med. 156: 546-549.

62. Abraham, W. M.; Welker, M.; Oliver, W., Jr.; Mingle, M.; Januszkiewicz, A. J.; Wanner, A.; Sackner, M. A. (1980) Cardiopulmonary effects of short-term nitrogen dioxide exposure in conscious sheep. Environ. Res. 22: 61-72.

63. Yamamoto, I.; Takahashi, M. (1984) Ultrastructural observations of rat lung exposed to nitrogen dioxide for 7 months. Kitasato Arch. Exp. Med. 57: 57-65.

64. Rombout, P. J. A.; Dormans, J. A. M. A.; Marra, M.; van Esch, G. J. (1986) Influence of exposure regimen on nitrogen dioxide-induced morphological changes in the rat lung. Environ. Res. 41: 466-480.

65. Suzuki, T.; Ikeda, S.; Kanoh, T.; Mizoguchi, I. (1986) Decreased phagocytosis and superoxide anion production in alveolar macrophages of rats exposed to nitrogen dioxide. Arch. Environ. Contam. Toxicol. 15: 733-739.

66. Chang, L.-Y.; Graham, J. A.; Miller, F. J.; Ospital, J. J.; Crapo, J. D. (1986) Effects of subchronic inhalation of low concentrations of nitrogen dioxide. I. The proximal alveolar region of juvenile and adult rats. Toxicol. Appl. Pharmacol. 83: 46-61.

67. Mochitate, K.; Takahashi, Y.; Ohsumi, T.; Miura, T. (1986) Activation and increment of alveolar macrophages induced by nitrogen dioxide. J. Toxicol. Environ. Health 17: 229-239.

68. Gardner, D. E.; Holzman, R. S.; Coffin, D. L. (1969) Effects of nitrogen dioxide on pulmonary cell population. J. Bacteriol. 98: 1041-1043.

69. Acton, J. D.; Myrvik, Q. N. (1972) Nitrogen dioxide effects on alveolar macrophages. Arch. Environ. Health 24: 48-52.

70. Foster, J. R.; Cottrell, R. C.; Herod, I. A.; Atkinson, H. A. C.; Miller, K. (1985) A comparative study of the pulmonary effects of NO_2 in the rat and hamster. Br. J. Exp. Pathol. 66: 193-204.

71. Schlesinger, R. B.; Driscoll, K. E.; Vollmuth, T. A. (1987) Effect of repeated exposures to nitrogen dioxide and sulfuric acid mist alone or in combination on mucociliary clearance from the lungs of rabbits. Environ. Res. 44: 294-301.

72. Frampton, M. W.; Smeglin, A. M.; Roberts, N. J., Jr.; Finkelstein, J. N.; Morrow, P. E.; Utell, M. J. (1989) Nitrogen dioxide exposure *in vivo* and human alveolar macrophage inactivation of influenza virus *in vitro*. Environ. Res. 48: 179-192.

73. Pinkston, P.; Smeglin, A.; Roberts, N. J., Jr.; Gibb, F. R.; Morrow, P. E.; Utell, M. J. (1988) Effects of *in vitro* exposure to nitrogen dioxide on human alveolar macrophage release of neutrophil chemotactic factor and interleukin-1. Environ. Res. 47: 48-58.

74. Aranyi, C.; Fenters, J.; Erhlich, R.; Gardner, D. (1976) Scanning electron microscopy of alveolar macrophages after exposure to oxygen, nitrogen dioxide, and ozone. EHP Environ. Health Perspect. 16: 180.

75. Dowell, A. R.; Kilburn, K. H.; Pratt, P. C. (1971) Short-term exposure to nitrogen dioxide: effects on pulmonary ultrastructure, compliance, and the surfactant system. Arch. Intern. Med. 128: 74-80.

76. Hoofman, R. N.; Kuper, C. F.; Appelman, L. M. (1988) Comparative sensitivity of histo-pathology and specific lung parameters in the detection of lung injury. JAT J. Appl. Toxicol. 8: 59-65.

77. Lefkowitz, S. S.; McGrath, J. J.; Lefkowitz, D. L. (1986) Effects of NO_2 on immune responses. J. Toxicol. Environ. Health 17: 241-248.

78. Fujimaki, H.; Shimizu, F. (1981) Effects of acute exposure to nitrogen dioxide on primary antibody response. Arch. Environ. Health 36: 114-119.

79. Fujimaki, H.; Shimizu, F.; Kubota, K. (1982) Effect of subacute exposure to NO_2 on lymphocytes required for antibody responses. Environ. Res. 29: 280-286.

80. Richters, A.; Damji, K. S. (1988) Changes in T-lymphocyte subpopulations and natural killer cells following exposure to ambient levels of nitrogen dioxide. J. Toxicol. Environ. Health 25: 247-256.

81. Damji, K. S.; Richters, A. (1989) Reduction in T lymphocyte subpopulations following acute exposure to 4 ppm nitrogen dioxide. Environ. Res. 49: 217-224.

82. Goings, S. A. J.; Kulle, T. J.; Bascom, R.; Sauder, L. R.; Green, D. J.; Hebel, J. R.; Clements, M. L. (1989) Effect of nitrogen dioxide exposure on susceptibility to influenza A virus infection in healthy adults. Am. Rev. Respir. Dis. 139: 1075-1081.

83. Frampton, M. W.; Finkelstein, J. N.; Roberts, N. J., Jr.; Smeglin, A. M.; Morrow, P. E.; Utell, M. J. (1989) Effects of nitrogen dioxide exposure on bronchoalveolar lavage proteins in humans. Am. J. Respir. Cell Mol. Biol. 1: 499-505.

84. Ehrlich, R.; Henry, M. C. (1968) Chronic toxicity of nitrogen dioxide: I. effect on resistance to bacterial pneumonia. Arch. Environ. Health 17: 860-865.

85. Ito, K. (1971) [Effect of nitrogen dioxide inhalation on influenza virus infection in mice]. Nippon Eiseigaku Zasshi 26: 304-314.

86. Ehrlich, R.; Findlay, J. C.; Fenters, J. D.; Gardner, D. E. (1977) Health effects of short-term inhalation of nitrogen dioxide and ozone mixtures. Environ. Res. 14: 223-231.

87. Parker, R. F.; Davis, J. K.; Cassell, G. H.; White, H.; Dziedzic, D.; Blalock, D. K.; Thorp, R. B.; Simecka, J. W. (1989) Short-term exposure to nitrogen dioxide enhances susceptibility to murine respiratory mycoplasmosis and decreases intrapulmonary killing of *mycolplasma pulmonis*. Am. Rev. Respir. Dis. 140: 502-512.

88. Gardner, D. E.; Miller, F. J.; Blommer, E. J.; Coffin, D. L. (1977) Relationships between nitrogen dioxide concentration, time, and level of effect using an animal infectivity model. In: Dimitriades, B., ed. International conference on photochemical oxidant pollution and its control: v. I; September 1976; Raleigh, NC. Research Triangle Park, NC: U. S. Environmental Protection Agency, Environmental Sciences Research Laboratory; pp. 513-525; EPA report no. EPA-600/3-77-001a. Available from: NTIS, Springfield, VA; PB-264232.

89. Gardner, D. E.; Miller, F. J.; Blommer, E. J.; Coffin, D. L. (1979) Influence of exposure mode on the toxicity of NO_2. EHP Environ. Health Perspect. 30: 23-29.

90. Gardner, D. E.; Graham, J. A.; Illing, J. W.; Blommer, E. J.; Miller, F. J. (1980) Impact of exposure patterns on the toxicological response to NO_2 and modifications by added stressors. In: Proceedings of the U.S.-USSR symposium on problems of environmental health; pp. 17-40.

91. Gardner, D. E.; Miller, F. J.; Illing, J. W.; Graham, J. A. (1982) Non-respiratory function of the lungs: host defenses against infection. In: Schneider, T.; Grant, L., eds. Air pollution by nitrogen oxides: proceedings of the US-Dutch international symposium; May; Maastricht, The Netherlands. New York, NY: Elsevier Scientific Publishing Company; pp. 401-415. (Studies in environmental science 21).

92. Graham, J. A.; Gardner, D. E.; Blommer, E. J.; House, D. E.; Menache, M. G.; Miller, F. J. (1987) Influence of exposure patterns of nitrogen dioxide and modifications by ozone on susceptibility to bacterial infectious disease in mice. J. Toxicol. Environ. Health 21: 113-125.

93. Jakab, G. J. (1987) Modulation of pulmonary defense mechanisms by acute exposures to nitrogen dioxide. Environ. Res. 42: 215-228.

94. Motomiya, K.; Ito, K.; Yoshida, A.; Idewara, S.; Otsu, Y.; Nakajima, Y. (1973) The effects of exposure to NO_2 gas on the infection of influenza virus of mouse - long term experiment in low concentration. Kankyo Kagaku Kenkyu Hokoku Chiba Daigaku 1: 27-33.

95. Miller, F. J.; Graham, J. A.; Raub, J. A.; Illing, J. W.; Menache, M. G.; House, D. E.; Gardner, D. E. (1987) Evaluating the toxicity of urban patterns of oxidant gases. II. Effects in mice from chronic exposure to nitrogen dioxide. J. Toxicol. Environ. Health 21: 99-112.

96. Pennington, J. E. (1988) Effects of automotive emissions on susceptibility to respiratory infections. In: Watson, A. Y.; Bates, R. R.; Kennedy, D., eds. Air pollution, the automobile, and public health. Washington, DC: National Academy Press; pp. 499-518.